

**Diffraction Grating-Based Encoded Micro-Particles
For Multiplexed Experiments**

Cross-Reference to Relate Applications

5 This patent application claims the benefit of U.S. Provisional Patent
Application Serial No. 60/405,087 (Cidra Docket No. CC-0429), filed on August 20,
2002; and U.S. Provisional Patent Application Serial No. 60/410,541 (Cidra Docket
No. CC-0543), filed on September 12, 2002, which are incorporated herein by
reference. Copending patent application Serial No. (CiDRA Docket No. CC-0648),
10 filed contemporaneously herewith, contains subject matter related hereto and is
incorporated herein by reference in its entirety.

Technical Field

15 This invention relates to optical identification, and more particularly to
diffraction grating-based encoded optical elements/micro-particles for performing
multiplexed experiments.

Background Art

20 A common class of experiments comprises mixing or reacting a labeled but
unknown hybrid analyte with a set of "probe" substances, which is known as a
multiplexed experiment. Multiplexing allows many properties of the analyte to be
probed in simultaneously (or in parallel). For instance, in a gene expression assay, the
"target" analyte, usually an unknown sequence of DNA, is labeled with a fluorescent
molecule to form the hybrid analyte. Each probe consists of short complementary
25 DNA sequences that will selectively bind to segments of the unknown DNA sequence
of the "target" analyte. The probes then are spatially separated and will fluoresce at
different levels depending on how well the unknown strand of DNA binds or
hybridizes to each probe. By knowing the DNA sequence of each probe, the
sequences in the unknown target can be evaluated.

Generally the probes are spatially separated to identify the probe and ultimately the “target” analyte using one of two approaches. The first approach separates the probes in a predetermined grid, where the probe’s identity is linked to its position on the grid. One example of this approach is high-throughput screening systems that utilize multi-well plates, where the substance in each well is known. Aother example is a spotted DNA microarray, where printed spots of oligomer DNA sequences are put in a predetermined spatial order on a substrate (usually a glass microscope slide).

A second approach of identifying the probe allows the probes to mix without any specific spatial position, which is often called the “random bead assay” approach. In this approach the probes are not attached to a substrate but are free to move (usually in a liquid medium). This approach has an advantage in that the analyte reaction can be performed in a solution by conventional wet-chemistry techniques, which gives the probes a better opportunity to interact with the hybrid analyte. This approach, however, requires that each probe be individually identifiable.

There are many known methods and substrate types that can be used for tagging or otherwise uniquely identifying individual probes. Known methods include using polystyrene latex spheres that are colored or fluorescently labeled. Other methods include using small plastic cans with a conventional bar code applied, or a small container that includes a solid support material and a radio-frequency tag.

The methods of uniquely identifying the probes, however, may be large in size, have a limited number of identifiable codes and/or formed of material not suitable to harsh environmental condition, such as high temperature and/or corrosive material.

Therefore, it would be desirable to provide probes that are very small, capable of providing a large number of unique codes (e.g., greater than 1 million codes), and/or have codes intrinsic to the probe which are resistant to harsh enviroments.

Summary of the Invention

Objects of the present invention include a diffraction grating-based encoded micro-particles that are coated with a substance for multiplexed experiments, which
5 are very small, capable of providing a large number of unique codes, and/or have codes intrinsic to the probe which are resistant to harsh environments.

The invention is a significant improvement over chip based assay and existing bead assay technology, as discussed above.

The foregoing and other objects, features and advantages of the present
10 invention will become more apparent in light of the following detailed description of exemplary embodiments thereof.

Brief Description of the Drawings

Fig. 1 is a side view of an optical identification element, in accordance with
15 the present invention.

Fig. 2 is a side view of an optical identification element illuminated from the side, in accordance with the present invention.

Fig. 3 is a flow chart of the method of attaching a substance to an optical identification element, performing an assay and analyzing the optical identification
20 element, in accordance with the present invention.

Fig. 4 is a side view of an optical identification element having a substance attached to the outer surface thereof, in accordance with the present invention.

Fig. 5 is a schematic view of a plurality of optical identification elements having different identification or codes and coated with different probe substances
25 disposed in a cell with a plurality of test substances, in accordance with the present invention.

Fig. 6 is a schematic view of plurality of optical identification elements after the performance of an assay, aligned in a plurality of grooves, disposed in a glass substrate, and a bead detector that scans each optical identification element for

determining the code and fluorescence of each optical identification element, in accordance with the present invention.

Fig. 7 is a side view of an optical identification element after the performance of an assay, and a bead detector that determines the code and fluorescence of the optical identification element, in accordance with the present invention.

Best Mode for Carrying Out the Invention

Referring to Fig. 1, an optical identification element 8 (microparticle or microbead) comprises a known optical substrate 10, having an optical diffraction grating 12 disposed (or written, impressed, embedded, imprinted, etched, grown, deposited or otherwise formed) in the volume of or on a surface of a substrate 10. The grating 12 is a periodic or aperiodic variation in the effective refractive index and/or effective optical absorption of at least a portion of the substrate 10.

The microbead or microparticle described herein is the same as that described in Copending patent application Serial No. (CiDRA Docket No. CC-0648), filed contemporaneously herewith, which is incorporated herein by reference in its entirety.

The substrate 10 has an inner region 20 where the grating 12 is located. The inner region may be photosensitive to allow the writing or impressing of the grating 12. The substrate 10 has an outer region 18 which does not have the grating 12 therein.

The grating 12 is a combination of a plurality of individual spatial periodic sinusoidal variations in the refractive index that are collocated along the length of the grating region 20 of the substrate 10, each having a spatial period (or pitch) Λ . The grating 12 (or a combination of gratings) represents a unique optically readable code, made up of bits. In one embodiment, a bit corresponds to a unique pitch Λ within the grating 12.

The grating 12 may also referred to herein as a composite or collocated grating. Also, the grating 12 may be referred to as a "hologram", as the grating 12

transforms, translates, or filters an input optical signal to a predetermined desired optical output pattern or signal.

5 The substrate 10 has an outer diameter D1 and comprises silica glass (SiO_2) having the appropriate chemical composition to allow the grating 12 to be disposed therein or thereon. Other materials for the optical substrate 10 may be used if desired. For example, the substrate 10 may be made of any glass, e.g., silica, phosphate glass, or other glasses, or made of glass and plastic, or solely plastic. For high temperature or harsh chemical applications, the optical substrate 10 made of a glass material is desirable. The optical substrate 10 may be any material capable of having the grating 10 12 disposed in the grating region 20 and that allows light to pass through it to allow the code to be optically read.

The optical substrate 10 with the grating 12 has a length L and an outer diameter D1, and the inner region 20 diameter D. The length L can range from small (about 1-1000 microns or smaller) to large (about 1.0 - 1000 mm or greater). In 15 addition, the outer dimension D1 can range from small (less than 1000 microns) to large (1.0 - 1000 mm and greater). Other dimensions and lengths for the substrate 10 and the grating 12 may be used. However, for experiment use, smaller size is typically best.

20 The grating 12 may have a length L_g of about the length L of the substrate 10. Alternatively, the length L_g of the grating 12 may be shorter than the total length L of the substrate 10.

Moreover, the size of any given dimension for the region 20 of the grating 12 may be less than any corresponding dimension of the substrate 10. For example, if the grating 12 has dimensions of length L_g , depth D_g , and width W_g , and the substrate 12 25 has dimensions of length L, depth D, and width W, the dimensions of the grating 12 may be less than that of the substrate 12. For a cylindrical grating region Thus, the grating 12, may be embedded within or part of a much larger substrate 12. Instead of rectangular dimensions or coordinates for size of the substrate 10, the element 8, or

the grating 12, other dimensions/coordinates for size may be used, e.g., polar or vector dimensions.

Also, the element 8 may be embedded or formed in or on a larger object for identification of the object. For example, a microscope slide or test tube can have an element 10 embedded therein or thereon.

The substrate 10 may have end-view cross-sectional shapes other than circular, such as square, rectangular, elliptical, clam-shell, D-shaped, or other shapes, and may have side-view sectional shapes other than rectangular, such as circular, square, elliptical, clam-shell, D-shaped, or other shapes. Also, 3D geometries other than a cylinder may be used, such as a sphere, a cube, a pyramid or any other 3D shape. Alternatively, the substrate 10 may have a geometry that is a combination of one or more of the foregoing shapes.

The dimensions, geometries, materials, and material properties of the substrate 10 are selected such that the desired optical and material properties are met for a given application. The resolution and range for the optical codes are scalable by controlling these parameters (discussed more hereinafter).

The substrate 10 may be coated with a polymer material or other material that may be dissimilar to the material of the substrate 10, provided that the coating on at least a portion of the substrate, allows sufficient light to pass transversely through the substrate for adequate optical detection of the code using side illumination.

Referring to Fig. 1, the outer region 18 is made of pure silica (SiO_2) and has a refractive index n_2 of about 1.458 (at a wavelength of about 1553 nm), and the inner grating region 20 of the substrate 10 has dopants, such as germanium and/or boron, to provide a refractive index n_1 of about 1.453, which is less than that of outer region 18 by about 0.005. Other indices of refraction n_1, n_2 for the grating region 20 and the outer region 18, respectively, may be used, if desired, provided the grating 12 can be impressed in the desired grating region 20. For example, the grating region 20 may have an index of refraction that is larger than that of the outer region 18 or grating region 20 may have the same index of refraction as the outer region 18 if desired.

The primary purpose of the outer region 18 (or region without the grating 12) of the substrate 10 is to provide mechanical or structural support for the inner grating region 20. Referring to Fig. 3, accordingly, the entire substrate 10 may comprise the grating 12, if desired. Referring to Fig. 4, alternatively the support portion may be completely or partially beneath, above, or along one or more sides of the grating region 20, such as in a planar geometry (Fig. 4), or a D-shaped geometry (Fig. 5), or other geometries. The non-grating portion 18 of the substrate 10 may be used for other purposes as well, such as optical lensing effects or other effects (discussed hereinafter).

Also, the end faces of the substrate 10 need not be perpendicular to the sides or parallel to each other.

The incident light 24 of a wavelength λ , e.g., 532 nm from a known frequency doubled Nd:YAG laser or 632nm from a known Helium-Neon laser, is incident on the grating 12 in the substrate 10. Any other input wavelength λ can be used if desired provided λ is within the optical transmission range of the substrate (discussed more hereinafter).

A portion of the input light 24 passes straight through the grating 12 as indicated by dashed lines 25. The remainder of the light 24 is reflected by the grating 12 and forms a plurality of beams 26-36, each having the same wavelength λ as the input wavelength λ and each having a different angle indicative of the pitches (Λ_1 - Λ_n) existing in the grating 12.

As discussed hereinbefore, the grating 12 is a combination of a plurality of individual spatial periods or pitches Λ of the refractive index variation along the substrate, each collocated at substantially the same location on the substrate 10 (discussed more hereinafter). The resultant combination of these individual pitches is the grating 12 comprising spatial periods (Λ_1 - Λ_n) each representing a bit in the code. Accordingly, the code is determined by which spatial periods (Λ_1 - Λ_n) exist (or do not exist) in a given composite grating 12. The code may also be determined by additional parameters as well, as discussed hereinafter.

The reflected light 26-36 passes through a lens 37, which provides focused light beams 46-56 which are imaged onto a CCD camera 60. Instead of or in addition to the lens 37, other imaging optics may be used to provide the desired characteristics of the optical image/signal onto the camera 60 (e.g., spots, lines, circles, ovals, etc.), depending on the shape of the substrate and input optical signals. Also, instead of a CCD camera other devices may be used to read/capture the output light.

Each of the individual spatial periods (Λ_1 - Λ_n) in the grating 12 is slightly different, thus producing an array of N unique diffraction conditions (or diffraction angles) discussed more hereinafter. When the element 8 is illuminated from the side, in the region of the grating 12, at the appropriate angle (discussed hereinafter), with a single input wavelength λ (monochromatic) source, the diffracted (or reflected) beams 26-36 are generated.

The beams 26-36 are imaged onto the CCD camera 60 to produce a pattern of light and dark regions representing a digital (or binary) code, where light = 1 and dark = 0 (or vice versa). The digital code may be generated by selectively creating individual index variations (or individual gratings) with the desired spatial periods Λ_1 - Λ_n . Other illumination, readout techniques, types of gratings, geometries, materials, etc. may be used as discussed in the aforementioned patent application.

Referring to Figs. 3 - 7, the substrate 10 of the optical identification element (or microbead) 8 may be functionalized by coating the substrate with a material of interest 50, which is then used in a chemical reaction or as an attractant for certain chemicals 52. This capability to uniquely encode a large number of microbeads 8 with a corresponding number of different substances or materials attached to each microbead enables these coated microbeads to be mixed with an unknown analyte 52 to perform a multiplexed experiment. The procedure 40 for performing such a multiplexed assay or experiment includes the steps of producing 42 the probe or microbead 8, as described hereinbefore, and functionalizing 44 the outer surface of the microbead 8 by coating/depositing it with a material 50 that will react in a predetermined way with other chemicals/substances 52. An assay is then performed

46 with a plurality of microbeads with different identification codes 49 at the same time. In step 48, the fluorescence of the microbeads 8 is analyzed, and the identification element 8 is read to determine the code of each microbead to thereby determine information about the chemical reaction.

5 In Fig. 4, a coated microbead 54 is shown, wherein the outer surface of the microbead 8 is coated with a material 50 (functionalized) and used in a chemical reaction or as an attractant for certain test material 52 (see Fig. 5). The coating material 50 comprises a probe molecule or compound 56 attached to the microbead 8 by a linker molecule or complex. The probe molecule 56 includes a molecular group
10 55 for attachment to the linker molecule 58 and a molecule/compound of interest 57, such as an Oligonucleitides (oligos), antibodies, peptides, amino acid strings, cDNA, RNA, chemicals, nucleic acid oliomers, polymers, biological cells, or proteins. For example, the probe molecule 50 may comprise a single strand of DNA (or portion thereof) and the test material 52 comprising at least one unknown single strand of
15 DNA. As shown, the probe molecule 56 is attached or adhered to the outer surface of the substrate 10 by a linker molecule or complex 58. In some instances, the molecule of interest 57 of the probe molecule 56 may be attached directly to the outer surface of the substrate 10, or directly synthesized (or grown) onto the surface of the microbead 8, such as via phosphoramidite chemistry. Examples of surface chemistry for the
20 microbeads 8 include Streptavidin/biotinylated oligos and Aldehyde/amine modified oligos. Further, the microbead may be coated with blocker of non-specific binding (e.g., salmon sperm DNA) to prevent bonding of molecules (e.g. DNA) to the non-functionalized surface 59 of the microbeads.

Referring to Fig. 5, a plurality of functionalized microbeads 54 may then be
25 placed within a cell or container 60 to perform an assay. As discussed in step 46 of Fig. 3, the functionalized microbeads 54 placed in the cell have different identification codes 49. Each identification code 49 corresponds to a unique molecule of interest 57. For example, all functionalized microbeads 54 disposed within the cell having an identification code of 12345678 is coated with a unique molecule of interest 52, while

all functionalized microbeads 54 having an identification code of 34128913 is coated with a different unique molecule of interest.

5 The test material or molecules 52, disposed within a solution, are then injected into the cell 60 and mixed with the functionalized microbeads 54. The test molecules may include a single type of unknown molecule, or in most cases, the test molecules
10 comprise a plurality of different unknown test molecules. During mixing of the solution of test molecules 52 and functionalized microbeads 54, the test molecules attach to the complementary molecules of interest 57, as shown for functionalized microbeads having codes 12345678, 51627719, and 99132614. For example as discussed hereinbefore, each coded functionalized microbead 8 has a unique
15 molecules of interest 57 attached thereto, such as a portion of a single strand of DNA. Similarly, the test molecules of the analyte comprise a plurality of unknown single strands of DNA. These test molecules 52 are also processed with a fluorescent, such as dyeing, such that the test molecules illuminate. As will be discussed hereinafter, the fluorescence of the test molecules 52 provide the means to identify, which
functionalized microbeads have a test molecule attached thereto.

Once the reaction or combining is complete, the functionalized microbeads 54 are rinsed off with a saline solution to clean off the uncombined test molecules 52. As shown in Fig. 6, the functionalized microbeads 54 may be placed in a tray 64 with
20 grooves 62 to allow the microbeads 54 to be aligned in a predetermined direction, such as that described in U.S. Patent Application Serial No. (Cidra Docket No. CC-0648), filed contemporaneously,, which is incorporated herein by reference. The grooves 62 may have holes (not shown) that provide suction to keep the microbeads 54 in position. Once aligned in the tray 64, the functionalized microbeads 54 are
25 individually scanned and analyzed by the bead detector 20.

As best shown in Fig. 7, each functionalized microbead 54 is detected for fluorescence and analyzed to determine the identification code 49 of the microbead 54. A light source (not shown) may be provided to luminate the microbeads 54. Once the fluorescent microbeads 54 are identified and knowing which single strand of

DNA was attached to each coded micribead 54, the bead detector 20 determines which single strands of DNA were present in the test material 52. As described hereinbefore, the bead detector 20 illuminates the microbead 54 and focuses light reflected by the diffraction grating 12 onto a CCD array or camera 31, whereby the code 49 of the microbead is determined. Secondly, the bead detector 20 includes a fluorescence detector 66 for measuring the fluorescence emanating from test molecules 52 attached to the element 8. The fluorescence meter 66 includes a lens 68 and optical fiber 70 for receiving and providing the fluorescence from the test molecules 52 to the fluorescence meter.

The invention may be used in many areas such as drug discovery, functionalized substrates, biology, proteomics, combinatorial chemistry, DNA analysis/tracking/sorting/tagging, as well as tagging of molecules, biological particles, matrix support materials, immunoassays, receptor binding assays, scintillation proximity assays, radioactive or non-radioactive proximity assays, and other assays, (including fluorescent, mass spectroscopy), high throughput drug/genome screening, and/or massively parallel assay applications. The invention provides uniquely identifiable beads with reaction supports by active coatings for reaction tracking to perform multiplexed experiments.

Some current techniques used in combinatorial chemistry or biochemistry are described in US Patent No. 6,294,327, entitled "Apparatus and Method for Detecting Samples Labeled With Material Having Strong Light Scattering Properties, Using Reflection Mode Light and Diffuse Scattering", issued Sept. 23, 2001 to Walton et al.; US Patent No. 6,242,180, entitled "Computer Aided Visualization and Analysis System for Sequence Evaluation", issued June 5, 2001, to Chee; US Patent No. 6,309,823 entitled "Arrays of Nucleic Acid Probes for Analyzing Biotransformation of Genes and Methods of Using the Same", Oct. 30, 2001, to Cronin et al.; US Patent No. 6,440,667, entitled "Analysis of Target Molecules Using an Encoding System"; US Patent No. 6,355,432, entitled "Products for Detecting Nucleic Acids"; US Patent No. 6,197,506, entitled "Method of Detecting Nucleic Acids"; US Pat No. 6,309,822,

entitled "Method for comparing copy number of nucleic acid sequences"; US Patent No. 5,547,839, entitled "Sequencing of surface immobilized polymers utilizing microfluorescence detection", which are all incorporated herein by reference to the extent needed to understand the present invention.

5 The invention can be used in combinatorial chemistry, active coating and functionalized polymers, as well as immunoassays, and hybridization reactions. The invention enables millions of parallel chemical reactions, enable large-scale repeated chemical reactions, increase productivity and reduce time-to-market for drug and other material development industries.

10 The microbeads 8 are inexpensive to manufacture and the identification codes 49 are easy and inexpensive to imprint into the microbeads. The codes are digitally readable and easily adapted to optical coding techniques. Thus, the optical readout is very simple and inexpensive to implement. The code is not affected by spot imperfections, scratches, cracks or breaks. In addition, splitting or slicing an element
15 axially produces more elements with the same code; therefore, when a bead is axially split-up, the code is not lost, but instead replicated in each piece. Unlike electronic ID elements, the elements of the present invention are not affected by nuclear or electromagnetic radiation.

20 The dimensions and geometries for any of the embodiments described herein are merely for illustrative purposes and, as such, any other dimensions may be used if desired, depending on the application, size, performance, manufacturing requirements, or other factors, in view of the teachings herein.

25 It should be understood that, unless stated otherwise herein, any of the features, characteristics, alternatives or modifications described regarding a particular embodiment herein may also be applied, used, or incorporated with any other embodiment described herein. Also, the drawings herein are not drawn to scale.

 Although the invention has been described and illustrated with respect to exemplary embodiments thereof, the foregoing and various other additions and

omissions may be made therein and thereto without departing from the spirit and scope of the present invention.